

## Epidemiologic characteristics associated with SARS-CoV-2 antigen-based test results, rRT-PCR cycle threshold values, subgenomic RNA, and viral culture results from university testing

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**Summary:** Quidel Sofia SARS Antigen FIA testing was more sensitive in symptomatic participants and specimens with lower cycle threshold values. Characteristics of tests, target population and patient characteristics should be considered in SARS-CoV-2 testing programs.

## Abstract

**Background:** Real-time reverse transcription polymerase chain reaction (rRT-PCR) and antigen tests are important diagnostics for SARS-CoV-2. Sensitivity of antigen tests has been shown to be lower than that of rRT-PCR; however, data to evaluate epidemiologic characteristics that affect test performance are limited.

**Methods:** Paired mid-turbinate nasal swabs were collected from university students and staff and tested for SARS-CoV-2 using both Quidel Sofia SARS Antigen Fluorescent Immunoassay (FIA) and rRT-PCR assay. Specimens positive by either rRT-PCR or antigen FIA were placed in viral culture and tested for subgenomic RNA (sgRNA). Logistic regression models were used to evaluate characteristics associated with antigen results, rRT-PCR cycle threshold (Ct) values, sgRNA, and viral culture.

**Results:** Antigen FIA sensitivity was 78.9% and 43.8% among symptomatic and asymptomatic participants respectively. Among rRT-PCR positive participants, negative antigen results were more likely among asymptomatic participants (OR 4.6, CI:1.3-15.4) and less likely among participants reporting nasal congestion (OR 0.1, CI:0.03-0.8). rRT-PCR-positive specimens with higher Ct values (OR 0.5, CI:0.4-0.8) were less likely, and specimens positive for sgRNA (OR 10.2, CI:1.6-65.0) more likely, to yield positive virus isolation. Antigen testing was >90% positive in specimens with Ct values <29. Positive predictive value of antigen test for positive viral culture (57.7%) was similar to that of rRT-PCR (59.3%).

**Conclusions:** SARS-CoV-2 antigen test advantages include low cost, wide availability and rapid turnaround time, making them important screening tests. The performance of antigen tests may vary with patient characteristics, so performance characteristics should be accounted for when designing testing strategies and interpreting results.

**Keywords:** COVID-19, SARS-CoV-2, RT-PCR, Antigen test, Epidemiology, Sofia SARS Antigen FIA

## Introduction

Antigen-based tests are increasingly used for testing for SARS-CoV-2, the virus that causes coronavirus disease 2019 (COVID-19), as they are readily available, low cost, and return results quickly [1, 6]. Rapid results can ensure quick identification of infectious persons and enable efficient isolation and contact tracing. Antigen tests are therefore useful screening tests, particularly in congregate settings [1-4]. However, in asymptomatic individuals some antigen tests have had reduced sensitivity compared to real-time reverse transcription-polymerase chain reaction (rRT-PCR) [6].

While rRT-PCR is considered the most sensitive test for virus nucleic acid detection, the presence of nucleic acid does not always indicate contagiousness [1]. Recovery of virus in culture from patient specimens is presumed to indicate active infection and a high likelihood of contagiousness [15, 16]. However, viral culture has low sensitivity, even for specimens with low rRT-PCR cycle threshold (Ct) values [7]. Further, the absence of culturable virus does not necessarily indicate absence of transmissible virus, and viral culture is not feasible in most diagnostic or screening settings [17]. While lower Ct values and the detection of subgenomic RNA (sgRNA) are associated with higher viral load and greater likelihood of positive viral culture from a specimen [7-14], these additional analyses are not typically available for diagnostic purposes.

While prior studies have examined participant or specimen characteristics associated with SARS-CoV-2 test results [10, 18-20], data on the association of epidemiologic characteristics with performance of antigen testing, sgRNA detection, and viral culture are limited. Here we build on an earlier report [6] to describe specimen and participant characteristics associated with the performance of the Quidel Sofia SARS Antigen Fluorescent Immunoassay (FIA); we assess performance in relation to Ct values from rRT-PCR assays, sgRNA test results, and viral culture, in asymptomatic and symptomatic participants.

## Methods

We collected paired mid-turbinate nasal swabs, demographics, symptom information, and exposure history using a standardized questionnaire from students, faculty, staff and other affiliates at University A in Wisconsin as previously described [6]. Although our earlier report included specimens from two universities, we limited this analysis to persons from University A because University B used a different rRT-PCR test than University A and Ct values were not comparable across the two tests. At University A, weekly SARS-CoV-2 antigen or rRT-PCR testing was required for students living on-campus; free testing was also available to students living off-campus, university staff, and other university-affiliated persons. All persons tested at University A's testing center during October 1–9, 2020 were eligible to participate. A convenience sample of persons completed a paper questionnaire at check-in and provided an additional swab. Individuals could participate more than once if tested on different days.

Mid-turbinate nasal swabs for antigen testing were collected, processed and analyzed according to the manufacturer's instructions using the Sofia 2 analyzer (Quidel Corporation, San Diego, CA)<sup>1</sup> [5], and results were reported as positive, negative, or invalid [5]. Mid-turbinate nasal swabs for rRT-PCR were collected and stored in Viral Transport Media at 4°C. rRT-PCR was performed using the CDC 2019-nCoV RT-PCR Diagnostic Panel for detection of SARS-CoV-2 [21], with Ct values reported for N1 and N2 gene regions of the nucleocapsid protein; Ct values <40 were considered positive. Specimens were reported as negative (no targets positive), inconclusive (only one target positive) or positive (both targets positive). Paired specimens with inconclusive or invalid results from either antigen or rRT-PCR testing were excluded. Viral culture [22] and subgenomic RNA testing using rRT-PCR was attempted on residual rRT-PCR specimens if either the rRT-PCR or paired antigen test was positive. Specimens were considered sgRNA-positive if positive for either subgenomic spike or nucleocapsid gene regions. Full methods are described in the supplementary materials.

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<sup>1</sup> Use of trade names and commercial sources is for identification only and does not imply endorsement by the U.S. Department of Health and Human Services or CDC.

Participants were considered symptomatic if they reported  $\geq 1$  symptom at specimen collection, and asymptomatic if they did not report any symptom at specimen collection. Asymptomatic rRT-PCR positive participants were followed up by telephone within eight weeks of testing and considered pre-symptomatic if they experienced symptoms following specimen collection (but remained classified as asymptomatic for all analyses).

Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were calculated for the Sofia SARS Antigen FIA, using the rRT-PCR result to define presence or absence of SARS-CoV-2 infection. A concordant positive result from both antigen test and rRT-PCR was considered a true-positive, a negative antigen test result and a positive rRT-PCR result was considered a false-negative, a positive antigen test result and a negative rRT-PCR result was considered a false-positive, and a negative antigen test and rRT-PCR result was considered a true-negative. Sensitivity and specificity were calculated comparing the Sofia SARS antigen FIA to rRT-PCR among quarantined persons stratified by symptom status. Sensitivity, specificity and the proportion of specimens with recovered virus were also calculated comparing the Sofia SARS antigen FIA to rRT-PCR positive specimens using alternative Ct value cutoffs between  $<17$  and  $<40$  for defining rRT-PCR positivity, stratified by symptom status. PPVs were calculated for antigen testing, rRT-PCR, and sgRNA compared to viral recovery; chi squared tests were performed to test for differences. Confidence intervals (CI) were calculated at the 95%-level using the exact binomial method.

To understand which specimen and participant characteristics were associated with different test results, we compared specimen characteristics (Ct value and sgRNA detection) and participant characteristics (sex, age, collection date, symptom status, specific symptoms and days since symptom onset) using Firth's logistic regression [23], chosen to minimize bias in maximum likelihood estimates due to rarity of events in some groups. We compared antigen negative to antigen positive specimens among rRT-PCR positive specimens, and antigen positive to antigen negative specimens among rRT-PCR negative specimens. Among rRT-PCR positive specimens,

we also compared specimens with Ct values  $<25$  to specimens with Ct values  $\geq 25$ , presence of sgRNA to absence of sgRNA, and culture positive to culture negative specimens. Participant and specimen characteristics were modeled using univariable analysis, then characteristics with p-values  $<0.1$  on univariable analysis were combined in a multivariable model (Supplementary materials). We performed statistical analyses using Stata (version 16.1; StataCorps) and R (version 4.0.2).

This investigation was reviewed by CDC and the Wisconsin Division of Health Services and was conducted consistent with applicable federal law and CDC policy as defined in 45 CFR 46.102(l)(2). The ethical review board at University A determined the activity to be non-research Public Health Surveillance.

## Results

### *Participant demographics*

We collected 1,058 paired nasal swabs: 54 (5.1%) were positive for SARS-CoV-2 by rRT-PCR, and 997 (94.2%) were negative; seven (0.7%) were inconclusive and excluded from analyses (Supplementary Figure 1). The 1,051 paired swabs included in analyses were collected from 995 participants: 897 (90.2%) students, 79 (7.9%) faculty or staff, and 19 (1.9%) other university affiliates. Fifty-two participants participated twice and two participated three times; no significant differences were observed when excluding multiple visits. Participant demographics are shown in Table 1. Eighty-eight (8.4%) paired swabs were from participants in quarantine after being exposed to someone with confirmed COVID-19. Two hundred and nineteen swabs (20.8%) were from symptomatic participants and 832 swabs (79.2%) were from asymptomatic participants (Supplementary Table 1).

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<sup>2</sup> See e.g., 45 C.F.R. part 46.102(l)(2), 21 C.F.R. part 56; 42 U.S.C. §241(d); 5 U.S.C. §552a; 44 U.S.C. §3501 et seq.

### *Antigen test performance by participant characteristics*

Among symptomatic participants, 14.2% (31/219) were positive by the antigen test: 96.8% (30/31) were true-positives and 3.2% (1/31) were false-positives. Eight (3.7%) specimens were false-negative, and all eight were collected within five days of symptom onset. Sensitivity of the Sofia SARS Antigen FIA compared to rRT-PCR for symptomatic participants was 78.9%, specificity was 99.4%, PPV was 96.8%, and NPV was 95.7% (Table 2). Among symptomatic quarantined participants, sensitivity and specificity were similar (80.0% and 100%, respectively).

Among asymptomatic participants, 2.5% (21/832) were antigen positive: 33.3% (7/21) were true-positives and 66.7% (14/32) were false-positives. Nine (1.1%) specimens were false-negative. Of the seven asymptomatic true-positives, two participants reported  $\geq 1$  symptom in the 14 days prior to testing (mean Ct value 23.5), two participants were pre-symptomatic, developing  $\geq 1$  symptom one or two days after specimen collection (mean Ct value 25.8), two participants reported no symptoms before or after testing (mean Ct value 25.4), and one could not be contacted (Ct value 24.2). Of the nine asymptomatic false-negatives, one participant tested positive by rRT-PCR one month earlier (Ct value 35.0), five were pre-symptomatic, developing  $\geq 1$  symptom a median of 2 days (range 0-7) after specimen collection (mean Ct value 33.0), and three reported no symptoms in the two weeks prior or four to eight weeks after testing (mean Ct value 35.5).

Sensitivity of the Sofia SARS Antigen FIA compared to rRT-PCR among asymptomatic participants was 43.8%, specificity was 98.3%, PPV was 33.3% and NPV was 98.9% (Table 2). Among asymptomatic quarantined participants, sensitivity and specificity were similar (60.0% and 94.4% respectively), PPV was 60.0%, and NPV was 94.4%.

Among rRT-PCR positive specimens, asymptomatic participants had higher odds of a false-negative result (odds ratio (OR) 4.5, 95% CI 1.3-15.4). Among rRT-PCR positive symptomatic participants, those reporting nasal congestion were significantly less likely to have a false-negative result on univariable analysis (Table 3 and Supplementary Table 2).

On univariable and multivariable analyses of rRT-PCR negative specimens, participants with specimens collected later during October 1–9, males, and participants  $\geq 25$  years were more likely to have a false-positive test result (Table 3 and Supplementary Table 3). Test kits from two lots were used, one during October 1–7, 2020 and one during October 7–9, 2020. All 15 (100%) false-positives occurred during October 7–9 in a single lot of Sofia SARS Antigen FIA tests across four analyzers and technicians; 53.3% (8/15) of false-positive tests were performed in one hour by one analyzer. In this instance, repeat antigen testing was offered to affected participants; six of eight participants were re-swabbed within one hour and received a negative test result on the second antigen test. All eight participants were asymptomatic and their initial paired swabs were rRT-PCR negative. No user error was identified. Removing these eight, specificity among asymptomatic participants increased from 98.4% to 99.3% and PPV increased from 33.3% to 53.8%.

#### *Antigen test performance by Ct value*

Among rRT-PCR positive specimens, reporting symptoms was associated with Ct values  $< 25$ . Among symptomatic participants, nasal congestion was the only symptom associated with Ct values  $< 25$  on univariable analysis (Table 3 & Supplementary Tables 4-5).

Among symptomatic participants, antigen test sensitivity peaked at 96.3% using a Ct cutoff of  $< 29$  (Figure 1). For asymptomatic participants, sensitivity peaked at 100% with a Ct cutoff of  $< 29$ . Specimens with higher Ct values were more likely to be false-negative (Table 3 and Supplementary Table 6) and all (6/6) positive rRT-PCR specimens with Ct values  $\geq 35$  had negative antigen results. When including both the presence of symptoms and Ct value in multivariable analysis, only Ct value remained significantly associated with a false negative result.

#### *Antigen test performance by sgRNA*

sgRNA was detected in 85.2% (46/54) of rRT-PCR positive specimens. Among rRT-PCR positive specimens, reporting symptoms was associated with sgRNA presence and specimens with detectable sgRNA were less likely to be false-negative (OR 0.01, 95% CI 0.001-

0.3) (Table 3 & Supplementary Tables 6-8). sgRNA was detected in all 37 true positives, 44% (4/9) of asymptomatic false-negatives and 62.5% (5/8) of symptomatic false-negatives. sgRNA was not detected in any (0/15) false-positive specimens.

*Antigen test performance by viral culture*

Virus was recovered from 46.4% (32/69) of rRT-PCR or antigen positive specimens (Supplementary Figure 2); 81.1% (30/37) of true-positive specimens, 11.8% (2/17) of false-negative specimens, and 0% (0/15) of false-positive specimens. Among symptomatic participants, virus was recovered in 83.3% (25/30) of true-positive specimens and 25.0% (2/8) of false-negative specimens. Among asymptomatic participants, virus was recovered from 71.4% (5/7) of true-positive specimens and 0% (0/9) false-negative specimens. Virus was isolated from specimens with Ct values ranging from 17.4-29.8; virus was isolated from all specimens with a Ct value <25 and from 18.5% (5/27) of specimens with a Ct value  $\geq$ 25.

On univariable analyses of rRT-PCR positive specimens, the odds of isolating virus in culture decreased with increasing Ct values (OR 0.5, 95% CI 0.4-0.7 for every unit increase in Ct value) and increased in samples with detectable sgRNA (OR 10.2, 95% CI 1.6-65.0) (Supplementary Tables 9-10). Symptomatic participants were more likely to be culture positive than asymptomatic participants (OR 5.0, 95% CI 1.5-17.1). When adjusting for both symptoms and Ct value, only Ct value remained significantly associated with virus isolation.

Among all participants, antigen test PPV for virus isolation was similar to rRT-PCR PPV for virus isolation ( $p=0.87$ , Table 4). When excluding the eight false positive specimens that occurred over one hour, antigen test PPV for virus isolation increased to 68.2% but was still not significantly different from rRT-PCR PPV for virus isolation ( $p=0.36$ ).

## Discussion

SARS-CoV-2 antigen tests allow rapid isolation of infected individuals. In this investigation, antigen test results were available within two hours after specimen collection, while rRT-PCR results were available within 3-5 days. Therefore, antigen test results provided vital information for early initiation of isolation and contact tracing procedures for COVID-19 cases. Among symptomatic participants, the Sofia SARS Antigen FIA had lower sensitivity (79%) compared to rRT-PCR and sensitivity (44%) was even lower in asymptomatic participants [6]. However, sensitivity was >90% when using a Ct cutoff <29, suggesting antigen tests may perform better on specimens with higher viral loads. Specimens with lower Ct values or sgRNA positive were also more likely to have virus isolated. In this population, antigen test PPV for virus isolation was similar to rRT-PCR PPV for virus isolation among both symptomatic and asymptomatic participants.

Antigen test results should be interpreted in the context of COVID-19 prevalence and testing frequency [1]. In this population, antigen test PPV for rRT-PCR positive specimens was higher for asymptomatic quarantined students, where prevalence of infection was increased. Additionally, as weekly screening testing was required for students residing on-campus, we did not see many false-negative results from individuals no longer considered infectious, likely due to removal of these individuals from the testing pool through early identification with serial testing. Most false negative results were from symptomatic or pre-symptomatic individuals, suggesting that serial testing strategies using antigen testing-only may fail to detect some early infections captured by rRT-PCR. These data could inform models to evaluate the frequency of serial testing strategies, which should account for test sensitivity to optimize detection of infectious persons [25]. Additionally, confirmatory nucleic acid amplification testing is recommended in symptomatic persons who test antigen-negative, and in asymptomatic persons who test antigen-positive when pretest probability is low [1, 6].

Patient and specimen characteristics may also affect antigen test performance. In this investigation, rRT-PCR positive specimens were more likely to be positive by Sofia SARS Antigen FIA if Ct values were lower, if participants reported symptoms, and among symptomatic participants reporting nasal congestion. Increased sensitivity in specimens with lower Ct values is consistent with findings from other antigen tests [26-28]. Likewise, upper respiratory symptoms have previously been correlated with low Ct values in COVID-19 patients [10]. Nasal congestion may be associated with increased viral replication in the nares [29], increasing the viral load in a mid-turbinate nasal specimen and the likelihood of a positive antigen test.

rRT-PCR positive specimens with lower Ct values or from symptomatic participants were more likely to be positive on virus culture and have detectable sgRNA. While Ct values are thought to inversely correlate with viral load and increase the likelihood of positive viral culture, this correlation is imperfect. Several factors influence Ct values, including specimen collection, assay variability, and analytical variables like genomic extraction efficiency and storage/temperature fluctuations. [10, 30, 31]. Also, rRT-PCR enzyme efficiencies across a range of RNA concentrations may not be linear for a qualitative rRT-PCR assay [32]. It may, therefore, be problematic to infer a relationship between a specimen's Ct value from a qualitative rRT-PCR test and a patient's viral load or contagiousness [33]. Additionally, virus culture has limited sensitivity compared to rRT-PCR during acute SARS-CoV-2 illness [7, 34, 35]. Thus, absence of isolated virus should not be interpreted to mean a person is not currently infectious.

Presence of symptoms was associated with positive sgRNA. While moderate agreement has been demonstrated between virus culture and sgRNA previously, and sgRNA may suggest actively replicating intermediaries in specimens collected within a week of symptom onset [9, 11-13], sgRNA in clinical specimens does not necessarily signify active virus replication [31]. In this investigation, the PPV of sgRNA with culture was slightly higher than those of both rRT-PCR and antigen testing with culture among asymptomatic persons. Therefore, sgRNA could be a

better marker of live virus than antigen tests or rRT-PCR but may depend on specimen quality [36]. Further research is needed to meaningfully interpret how sgRNA presence relates to transmissible virus.

This investigation has several limitations. Participants were predominantly young adults with ongoing serial testing, potentially limiting generalizability to other populations. Associations with false-positive results may have been influenced by groups of males and staff members testing at the same time. As we did not attempt virus isolation on antigen and rRT-PCR negative specimens, only PPV was reported as a measure of agreement between antigen test, rRT-PCR, sgRNA and culture. The rRT-PCR assay used in this investigation is intended for the qualitative detection of nucleic acid and linearity across multiple virus concentrations was not formally established. Finally, this investigation evaluated the Sofia SARS Antigen FIA and cannot be generalizable to other FDA-authorized SARS-CoV-2 antigen tests.

In this investigation antigen tests were less sensitive than rRT-PCR but offered rapid turnaround time, had similar PPV for culture positive specimens to rRT-PCR, and identified all asymptomatic culture positive specimens. Antigen testing performed better among symptomatic participants, participants with nasal congestion and specimens with lower Ct values. Specimens with lower Ct values and from symptomatic participants were also more likely to have virus isolated or have detectable sgRNA. SARS-CoV-2 antigen test advantages include low cost, wide availability and rapid turnaround time, making them important screening tests; however, a negative test result only means that SARS-CoV-2 was not detected at the time of testing. Serial antigen testing, along with wearing masks and social distancing, as a part of a mitigation strategy provided a rapid method of identifying some, but not all, persons infected with SARS-CoV-2 in this investigation.

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Table 1: Characteristics of participants providing nasal swabs (N=1,051<sup>a</sup>), by results for SARS-CoV-2 real-time reverse transcription-polymerase chain reaction (rRT-PCR) and Sofia SARS Antigen Fluorescent Immunoassay testing at a university in Wisconsin, September-October, 2020

	rRT-PCR positive specimens (n=54)		rRT-PCR negative specimens (n=997)		Total (n=1,051 <sup>a</sup> ) N (%)
	Antigen positive (TP) (n=37) N (%)	Antigen negative (FN) (n=17) N (%)	Antigen positive (FP) (n=15) N (%)	Antigen negative (TN) (n=982) N (%)	
<b>Sex</b>					
Male	15 (40.5)	8 (47.1)	12 (80.0)	398 (40.5)	433 (41.2)
Female	22 (59.5)	9 (52.9)	3 (20.0)	584 (59.5)	618 (58.8)
<b>Age</b>					
15–24 years <sup>b</sup>	33 (89.2)	15 (88.2)	10 (66.7)	866 (88.2)	924 (87.9)
≥ 25 years	4 (10.8)	2 (11.8)	5 (33.3)	116 (11.8)	127 (12.1)
<b>Race/Ethnicity<sup>c</sup></b>					
White	30 (81.1)	16 (94.1)	12 (80.0)	829 (84.4)	887 (84.4)
Hispanic/Latino	5 (13.5)	0 (0)	1 (6.7)	51 (5.2)	57 (5.4)
Black/African-American	0 (0)	1 (5.9)	1 (6.7)	23 (2.3)	25 (2.4)
Asian/Pacific Islander	0 (0)	0 (0)	0 (0)	41 (4.2)	41 (3.9)
American Indian/Alaska Native	0 (0)	0 (0)	0 (0)	3 (0.3)	3 (0.3)
Multiple races	0 (0)	0 (0)	1 (6.7)	30 (3.1)	31 (2.9)
Unknown	2 (5.4)	0 (0)	0 (0)	5 (0.5)	7 (0.7)
<b>University status</b>					
Student	33 (89.2)	16 (94.1)	12 (80.0)	886 (90.2)	947 (90.1)
Faculty or staff	4 (10.8)	1 (5.9)	3 (20.0)	74 (7.5)	82 (7.8)
Other affiliate <sup>d</sup>	0 (0)	0 (0)	0 (0)	14 (1.4)	14 (1.3)
Unknown	0 (0)	0 (0)	0 (0)	8 (0.8)	8 (0.7)
<b>Quarantine status</b>					
Quarantined at time of sample collection	15 (40.5)	5 (29.4)	2 (13.3)	66 (6.7)	88 (8.4)
Time between quarantine initiation to sample collection, median days (interquartile range)	1 (1-6)	3 (1-5)	0.5 (0-1)	2 (1-7)	2 (1-6)
<b>Reported prior symptoms</b>					
No symptoms in past 14 days	9 (24.3)	12 (70.6)	15 (100)	789 (80.3)	825 (78.5)

≥1 symptom in past 14 days	28 (75.7)	5 (29.4)	0 (0)	193 (19.7)	226 (21.5)
<b>Reported current symptoms</b>					
No current symptoms	7 (18.9)	9 (52.9)	14 (93.3)	802 (81.7)	832 (79.2)
≥1 current symptom	30 (81.1)	8 (47.1)	1 (6.7)	180 (18.3)	219 (20.8)

TP = True-positive; FN = False-negative; FP = False-positive; TN = True-negative

<sup>a</sup> Includes 52 participants who presented twice for testing and 2 participants who participated three times, and were included more than once in the analysis.

<sup>b</sup> One 15-year-old child of a university staff member. All other participants were ≥17 years.

<sup>c</sup> Non-Hispanic ethnicity represented for all White, Black/African-American, Asian/Pacific Islander, American Indian/Alaska Native, Other /multiple races.

<sup>d</sup> “Other affiliates” were participants who did not mark “student” or “staff” on the questionnaire (they selected “other” or did not respond); the majority of these individuals were family members of staff.

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Table 2: Sensitivity, specificity, positive predictive value, and negative predictive value of Sofia SARS Antigen Fluorescent Immunoassay compared with real-time reverse transcription-polymerase chain reaction (rRT-PCR) among asymptomatic and symptomatic participants overall and in quarantine at a university in Wisconsin, September-October 2020

	Real-time RT-PCR result, no.			Test evaluation % (95% CI)		
	Positive	Negative	Total			
<b>Symptomatic (N=219)<sup>a</sup></b>	Ag Positive	30	1	31	Sensitivity	78.9 (62.7–90.4)
	Ag Negative	8	180	188	Specificity	99.4 (96.9–100)
	Ag Total	38	181	219	Positive predictive value	96.8 (83.3–99.9)
					Negative predictive value	95.7 (91.8–98.1)
<b>Symptoms meeting CSTE clinical criteria<sup>b</sup> (N=141)</b>	Ag Positive	26	1	27	Sensitivity	76.5 (58.8–89.3)
	Ag Negative	8	106	114	Specificity	99.1 (94.9–100)
	Ag Total	34	107	141	Positive predictive value	96.3 (81.0–99.9)
					Negative predictive value	93.0 (86.6–96.9)
<b>Symptomatic and in quarantine (N=47)</b>	Ag Positive	12	0	12	Sensitivity	80.0 (51.9–95.7)
	Ag Negative	3	32	35	Specificity	100 (89.1–100)
	Ag Total	15	32	47	Positive predictive value	100 (73.5–100)
					Negative predictive value	91.4 (76.9–98.2)
<b>Asymptomatic (N=832)</b>	Ag Positive	7	14	21	Sensitivity	43.8 (19.8–70.1)
	Ag Negative	9	802	811	Specificity	98.3 (97.1–99.1)
	Ag Total	16	816	832	Positive predictive value	33.3 (14.6–57.0)
					Negative predictive value	98.9 (97.9–99.5)
<b>Asymptomatic<sup>c</sup> (N=824)</b>	Ag Positive	7	6	13	Sensitivity	43.8 (19.8–70.1)
	Ag Negative	9	802	811	Specificity	99.3 (98.4–99.7)
	Ag Total	16	808	824	Positive predictive value	53.8 (25.1–80.8)
					Negative predictive value	98.9 (97.9–99.5)
<b>Asymptomatic and in quarantine</b>	Ag Positive	3	2	5	Sensitivity	60.0 (14.7–94.7)
					Specificity	94.4 (81.3–99.3)

<b>(N=41)</b>	Ag Negative	2	34	36	Positive predictive value	60.0 (14.7–94.7)
	Ag Total	5	36	41	Negative predictive value	94.4 (81.3–99.3)

Ag = Antigen test; CI = confidence interval; CSTE = Council of State and Territory Epidemiologists

<sup>a</sup> One or more symptoms reported.

<sup>b</sup> Participant reported symptoms meeting the Council for State and Territorial Epidemiologists (CSTE) clinical criteria for COVID-19

([https://cdn.ymaws.com/www.cste.org/resource/resmgr/ps/positionstatement2020/Interim-20-ID-02\\_COVID-19.pdf](https://cdn.ymaws.com/www.cste.org/resource/resmgr/ps/positionstatement2020/Interim-20-ID-02_COVID-19.pdf))

<sup>c</sup> Excluding the eight false positive results that occurred in one hour

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Table 3: Univariable odds ratios (ORs) and multivariable adjusted odds ratios (aORs) of specimen and participant characteristics that were statistically significant. (NA = Not applicable, multivariable regression not done because only one variable in univariable analysis had a p-value <0.1)

<b>1a. Characteristics associated with antigen negative test among rRT-PCR positive specimens</b>				
	<b>FN (n=17) No (%)</b>	<b>TP (n=37) No (%)</b>	<b>OR (95% CI)</b>	<b>aOR (95% CI)</b>
Ct Value	32.3 <sup>a</sup>	23.7 <sup>a</sup>	1.5 (1.2-1.9) <sup>b</sup>	1.5 (1.2-1.9)
Among symptomatic participants	(n=8)	(n=30)		
Nasal congestion	2 (25.0)	22 (73.3)	0.1 (0.03-0.8)	NA
<b>1b. Characteristics associated with antigen positive test among rRT-PCR negative specimens</b>				
	<b>FP (n=15) No (%)</b>	<b>TN (n=982) No (%)</b>	<b>OR (95% CI)</b>	<b>aOR (95% CI)</b>
Later collection date	-	-	3.6 (1.9-6.9)	3.7 (1.9-7.3)
Female (ref=Male)	3 (20.0)	584 (59.5)	0.2 (0.1-0.6)	0.2 (0.1-0.8)
≥25 years (ref=15-24years)	5 (33.3)	116 (11.8)	3.9 (1.4-11.1)	4.9 (1.6-15.7)
<b>1c. Characteristics associated with Ct value ≤25 among rRT-PCR positive specimens</b>				
	<b>CT&lt;25 (n=27) No (%)</b>	<b>CT ≥25 (n=27) No (%)</b>	<b>OR (95% CI)</b>	<b>aOR (95% CI)</b>
≥1 symptom	23 (85.2)	15 (55.6)	4.2 (1.2-14.8)	NA
Among symptomatic participants	(n=23)	(n=15)		
Nasal congestion	18 (78.3)	6 (40.0)	4.9 (1.2-19.5)	NA
<b>1d. Characteristics associated with detection of sgRNA among rRT-PCR positive specimens</b>				
	<b>sgRNA (n=46) No (%)</b>	<b>No sgRNA (n=8) No (%)</b>	<b>OR (95% CI)</b>	<b>aOR (95% CI)</b>
≥1 symptom	35 (76.1)	3 (37.5)	4.9 (1.1-21.7)	NA
<b>1e. Characteristics associated with virus recovery among rRT-PCR positive specimens</b>				
	<b>Positive (n=32) No (%)</b>	<b>Negative (n=22) No (%)</b>	<b>OR (95% CI)</b>	<b>aOR (95% CI)</b>
Ct Value	22.7 <sup>a</sup>	31.8 <sup>a</sup>	0.5 (0.4-0.7)	0.5 (0.4-0.8)

<sup>a</sup> Mean

<sup>b</sup> Odds ratio is for higher Ct value: the odds of a false negative result is 1.5 times for every unit increase in Ct value

FN=False-negative, TP=True-positive, FP=False-positive, TN=True-negative

Table 4: Positive predictive value (PPV) of antigen test for virus isolation, real-time reverse transcription-polymerase chain reaction (rRT-PCR) for virus isolation, and subgenomic RNA (sgRNA) for virus isolation

	PPV for virus isolation (95% CI)			
	Antigen	Antigen <sup>a</sup>	rRT-PCR	sgRNA
Overall	57.7% (43.2%-71.3%)	68.2% (52.4%-81.4%)	59.3% (45.0%-72.4%)	67.4% (52.0%-80.5%)
Symptomatic	80.6% (62.5%-92.5%)	80.6% (62.5%-92.5%)	71.1% (54.1%-84.6%)	74.3% (67.4%-87.5%)
Asymptomatic	23.8% (8.2%-47.2%)	38.5% (13.9%-68.4%)	31.3% (11.0%-58.7%)	45.5% (16.7%-76.6%)

<sup>a</sup>Excluding 8 false-positive specimens that occurred in one hour

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Figure 1: Sensitivity and specificity of SARS-CoV-2 Sofia Antigen FIA compared to rRT-PCR and percent virus recovered in culture by cycle threshold (Ct) value cutoffs in specimens from symptomatic (N=219) and asymptomatic (N=832) participants.

\* Excluding the suspected 8 false positive results that occurred within one hour (N=824). All other results presented in this figure are unaffected by this exclusion.

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Figure 1

